AP Enzyme Kinetics Lab

In this lab you will first record a baseline value for the rate at which the enzyme peroxidase converts hydrogen peroxide into water & oxygen. This baseline will establish the rate of the enzyme under standard conditions. Each group/member will then be assigned a variable to test the effects of on the enzyme.
You will use a tool called a spectrophotometer which measures color change. The reaction between peroxide and peroxidase will be easily visible by adding an indicator called guaiacol which turns from clear to brown during the reaction. This change in color will be measured by the spectrophotometer and provide a value called absorbance. Absorbance refers to how much light is absorbed by a solution in the spectrophotometer so as the solution becomes darker, the absorbance value will increase.

The variables you will test are as follows

2x enzyme	1/2x enzyme	2x substrate	1/2x substrate
1/4 x substrate	Higher pH (pH 8)	Lower pH (4)	NaCl solution

Blank Procedures
1. Add 2mL of the "S" tube (see below) to a cuvette.
2. Place into spectrophotometer.
3. Replace the "S" mixture back into the test tube.

Baseline Procedures
1. In a test tube "S" add 7mL H_2O, 1mL H_2O_2 and 0.5mL guaiacol.
2. In a second test tube "E" add 7mL H_2O and 1.5mL peroxidase enzyme.
3. Add 1mL of tube "S" contents into a cuvette then carefully place the cuvette into the Spec.
4. CAREFULLY add 1 mL of tube "E" contents in the cuvette and hit the start button on the Spec.

Table 1: Baseline Absorbance

Time (s)	Absorbance (nm)
0	
30	
60	
90	
120	
150	
180	
210	
240	
270	
300	

Experimental Procedures (Be sure to calibrate (blank) before each trial)

Use table 2 below to determine the proper amount of solutions to add to your tubes.

Table 2: Different variable volumes for reaction tubes

Tube 1	2x enz	1/2 enz	2x sub	1/2 sub	1/4 sub	Low pH	High pH	NaCl
Peroxide	1mL	1mL	2mL	0.5ml	0.25mL	1mL	1mL	1mL
Water	7mL	7mL	6mL	7.5mL	7.75mL	2mL	2mL	2mL
Guaiacol	0.5mL	0.5mL	0.5mL	0.5mL	0.5mL	0.5mL	0.5mL	0.5mL
8 buffer	X	X	X	X	X	X	5mL	X
4 buffer	X	X	X	X	X	5mL	X	X
NaCl	X	X	X	X	X	X	X	5mL
Tube 2	2x enz	1/2 enz	2x sub	1/2 sub	1/4 sub	Low pH	High pH	NaCl
Enzyme	3mL	0.75mL	1.5ml	1.5ml	1.5ml	1.5ml	1.5ml	1.5mL
Water	5.5mL	7.75mL	7mL	7mL	7mL	7mL	7mL	7mL

2x enzyme

Time (s)	Absorbance (nm)
0	
30	
60	
90	
120	
150	
180	
210	
240	
270	
300	

1/2 enzyme

Time (s)	Absorbance (nm)
0	
30	
60	
90	
120	
150	
180	
210	
240	
270	
300	

2x substrate

Time (s)	Absorbance (nm)
0	
30	
60	
90	
120	
150	
180	
210	
240	
270	
300	

1/2 substrate

Time (s)	Absorbance (nm)
0	
30	
60	
90	
120	
150	
180	
210	
240	
270	
300	

1/4 substrate

Time (s)	Absorbance (nm)
0	
30	
60	
90	
120	
150	
180	
210	
240	
270	
300	

pH 4

Time (s)	Absorbance (nm)
0	
30	
60	
90	
120	
150	
180	
210	
240	
270	
300	

pH 8

Time (s)	Absorbance (nm)
0	
30	
60	
90	
120	
150	
180	
210	
240	
270	
300	

NaCl

Time (s)	Absorbance (nm)
0	
30	
60	
90	
120	
150	
180	
210	
240	
270	
300	

You will generate **7 total graphs**:

1. **Baseline & 2 other enzyme concentrations** *as a function of time* (3 lines on single graph)
2. **Enzyme rate** *as a function of enzyme concentration* (1 line)
3. **Baseline & 3 other substrate concentrations** *as a function of time* (4 lines on single graph)
4. **Enzyme rate** *as a function of substrate concentration* (1 line)
5. **Baseline & 2 other pH levels** *as a function of time* (3 lines on single graph)
6. **Enzyme rate** *as a function of pH* (3 lines on single graph)
7. **Baseline & NaCl** *as a function of time* (2 lines on single graph)

Analysis

1. **Draw conclusions** about the variables - varying enzyme concentration, varying substrate concentration & varying pH level, adding an ionic compound (NaCl)
***Explain why the changes occurred on a **molecular level** using the terms active site, substrate, denaturation and Vmax where appropriate.

2. **Predict** the effects of varying temperature on enzyme activity and explain in terms of molecular changes to the enzyme.

One question not addressed is how inhibitors affect enzymes. The graph below shows typical results for comparing a baseline with competitive and non-competitive inhibitors. Some terms are defined below.

Vmax = maximum velocity of the enzyme; used to determine *maximum* rate of the enzyme as a function of increasing substrate concentration.

1/2 Vmax = Half the value of Vmax; used as a more accurate measure of enzyme's *steadiest rate* as a function of increasing substrate concentration.

Km = The **substrate concentration** at the **1/2 Vmax value** (Used to determine how *efficiently* the enzyme is converting substrate to product.

[S] = Concentration of the substrate; used to determine how the enzyme functions from low to high levels of substrate.

3. **Refine** our lab to include a means of determining whether a substance would be a competitive inhibitor or a non-competitive inhibitor. Be sure to explain required lab procedures and analysis procedures in order to determine this (hint – use the graph below for analysis!) Also, **explain** on a molecular level why competitive inhibitors have different Km and same Vmax as a baseline BUT non-competitive inhibitors have a different Vmax and same Km as a baseline.